

## Effect of honey: Inhibiting *Escherichia coli* and enhancing *Klebsiella pneumoniae* and *Proteus mirabilis*

Effect of honey on bacterial growth

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### Abstract

**Aim:** As sugar is the major component of honey, it may support bacterial growth in addition to the antibacterial property reported. The study was conducted to see the effect of different types of mono-floral honey on the isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*.

**Materials and Methods:** The in-vitro experimental study included nine different types of honey and five bacterial isolates. The floral origin of honey types was confirmed by microscopic observation of pollens, and bacterial isolates were identified using standard biochemical tests. Each honey type was diluted in 2-fold volume by volume in sterile distilled water to make concentrations 50%, 25%, 12.5%, and 6.25%, and their effect on the bacterial growth was studied using an agar well diffusion assay.

**Results:** All honey types had an inhibitory effect on *E. coli*. At 50% and 25% concentrations Sidr, Majrah Baldee, and Talha showed an inhibitory effect on *K. pneumoniae*, whereas Sidr Peshawri, Majrah Baldi, and Talha showed the inhibitory effect on *P. mirabilis*. The rest of concentrations of all honey types studied had growth-enhancing effect on the isolates of *K. pneumoniae* and *P. mirabilis*.

**Discussion:** Growth-enhancing effect of all honey types on *Klebsiella* and *Proteus* isolates is an important feature of this study. The results of the inhibitory effect pattern indicate a combined antimicrobial potential of honey ingredients. The varied effects on growth are clue to the prebiotic nature of honey.

### Keywords

Inhibitory effect; Enhancing effect; Honey

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## Introduction

Honey is a boon of nature produced by *Apis mellifera* bees [1]. Although the exact composition of honey varies as per the botanical source of origin [2], it contains a major proportion of sugar and other minor components such as amino acids, proteins, enzymes, organic acids, vitamins, minerals, volatile substances, and polyphenols [3]. The use of honey in therapy has been known for hundreds of years in global history [4]. Its clinical applications and recommendations are found in various religious literatures [4]. The Holy Quran is an Islamic religious book that clearly mentions that honey heals people in Chapter 16, Verse 69. It is being employed all over the world as complementary and alternative medicine (CAM) for the treatment of various diseases [3-5]. In Saudi Arabia, it has been reported that 14–73% of the CAM practices use honey in their therapy [5]. Despite being used in and well known for therapeutic remedies, honey still requires enough scientific support to secure a place in modern medicine [6, 7]. With the first notable study on honey for antibacterial potential in 1892 [8], the antimicrobial effect of honey can be seen in the literature search as the common focus of microbiological study [9-13]. Although the antimicrobial property of honey has been reported to be mainly due to its hydrogen peroxide content [10, 14-16], the rise of research studies on the activity of other honey components focusing on antimicrobial mode of action is observed not more than two and a half decades old [7]. As sugar is the major component of honey, it may support bacterial growth in addition to the antibacterial property reported. The present study has included various types of mono-floral unprocessed raw honey samples and aimed to observe their effects on selected bacterial isolates.

## Material and Methods

The in vitro experimental study was carried out from October 2016 to January 2017 in the microbiology laboratory of Al-Ghad International College of Applied Medical Sciences, Abha, Saudi Arabia. Ethical approval was sought from the institutional ethical committee, but as there was no involvement of human and animal subjects or the clinical samples, it was deemed not necessary for the research.

### Types of Honey and their processing

Nine different types of unprocessed natural raw honey samples were obtained from a local beekeeper maintaining apiculture in the outskirts of Abha under Aseer Province, Kingdom of Saudi Arabia, including one type of honey from Egypt. The respective types of honey were collected in sterile screw-capped tubes labeled with a specific code. The types of honey used in the study were Sidr (Si), Barseem (Bm), Taghtheyah (Tg), Sidr peshawari (Sp), Majra Baldee (Mb), Samrah (Sm), Tualha (TI), Shawkah (Sw) and Egypt orange honey (Eg) (Table 1). The tubes were kept at room temperature in the dark in the laboratory. The botanical origin of honey samples was determined by microscopic pollen analysis, and the floral origin was confirmed by 50% pollen dominance [17]. Initially, 50% (v/v) solution of honey was made in sterile distilled water. The respective solutions of honey were filtered using syringe filters of 0.45 µ pore size.

### Bacterial isolates and their identification

Cultures of five bacterial isolates were obtained from the microbiology laboratory of Aseer Central Hospital, Abha, with selection criteria including only those isolates known in the hospital microbiology laboratory to be pan-sensitive to the panel of antimicrobials. The isolates collected were *Escherichia coli* (n=2), *Klebsiella pneumoniae* (n=2), and *Proteus mirabilis* (n=1). The isolates were sub-cultured onto nutrient agar, blood agar and, MacConkey's agar plates. Gram staining, capsule staining (for *Klebsiella*) and the standard set of biochemical tests were performed to identify the bacterial isolates. The pan-sensitivity of the isolates to antimicrobials was confirmed using the standard Kirby-Bauer disk diffusion test for antibiotics recommended by the Clinical and Laboratory Standards Institute (CLSI) 2010 for *E. coli*, *K. pneumoniae* and *P. mirabilis*.

### Effect of Honey

#### Making serial dilutions of honey

Each honey type was serially diluted two-fold with sterile distilled water in four screw-capped sterile tubes to obtain concentrations of 50%, 25%, 12.5%, and 6.25% volume by volume.

#### Agar well diffusion assay for honey

A screening assay using well diffusion was carried out with some minor modifications [18]. Nutrient agar was prepared according to the manufacturer (HiMedia) instructions and dispensed aseptically in sterile 90 mm disposable Petri dishes to a height of about 4 mm. The plates were kept to cool for proper solidifying of the medium. The entire surface of the agar medium was inoculated with 0.5 McFarland bacterial suspension using sterile cotton swabs. After inoculation, four wells of 7 mm diameter were cut into the surface of the agar using a sterile well borer. After labeling the wells on the back of the plate, each well is filled with 100 µl of the respective concentrations of honey. The plates were incubated at 37°C for 24 hours. The diameter of the zone, including the diameter of the well, was recorded using ruler scale in millimeter. The zone with less or no growth was considered as the inhibitory zone, whereas the zone with more growth as growth-enhancing zone. In cases where there was growth around the wells similar to the lawn of growth on the same agar surface, honey was deemed ineffective against bacterial strain.

## Results

### Growth-Inhibitory Effect

Mean±SD of inhibition zone diameters of all honey types on both isolates of *E. coli* is presented at concentrations of 50%, 25%, 12.5% and 6.25% in Table 2. All the types of honey had an inhibitory effect on the isolates of *E. coli* (Figure 1, Table 2). Si, Mb and TI types of honey showed an inhibitory effect at 50% and 25% concentrations on both the isolates of *Klebsiella pneumoniae* (Figure 2, Table 2), while Sp, Mb and TI showed inhibition over *Proteus mirabilis* at 50% and 25% concentrations (Figure 3, Table 2).

### Growth-Enhancing Effect

None of the honey types showed growth-enhancing effect on either isolate of *E. coli* (Table 2). All nine types of honey had growth-enhancing effect on the isolates of *Klebsiella pneumoniae* and *Proteus mirabilis* (Table 2).

**Statistical Analysis**

The data were tabulated in an Excel sheet and statistically presented using mean and standard deviation (Mean±SD).

**Table 1.** Types of honey samples in the study

Local Name	Code used	Botanical origin	Geographic origin
Sidr	Si	<i>Ziziphus spina-christi</i>	Aseer
Barseem	Bm	<i>Trifolium alexandrium</i>	Aseer
Taghdeyah	Tg	Artificial sugar (Sucrose) solution	Aseer
Sidr Peshawari	Sp	<i>Ziziphus spina-christi</i>	Aseer
Majra Baldee	Mb	<i>Hypoestes forskoolii</i>	Aseer
Samrah	Sm	<i>Acacia tortilis</i>	Aseer
Tualha	Tl	<i>Acacia gerrardii</i>	Aseer
Shawkah	Sw	<i>Acacia nilotica</i>	Aseer
Egypt Orange Honey	Eg	<i>Citrus species</i>	Egypt

**Table 2.** Effect of honey types on the bacterial isolates (n = 5)

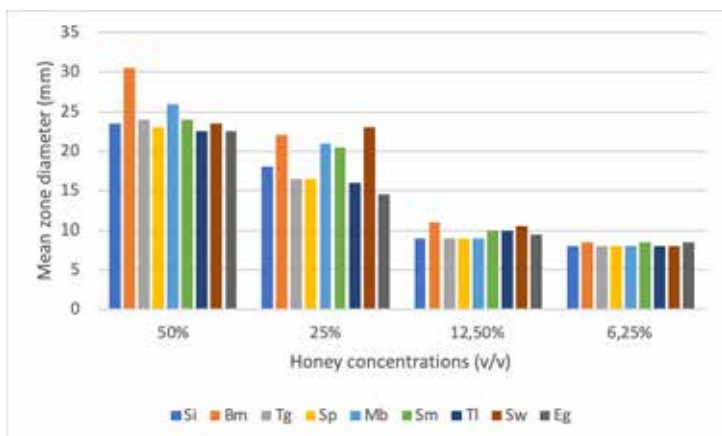
Effect	Organisms	Zone diameter (mm) with honey concentrations			
		50%	25%	12.50%	6.25%
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
Inhibitory	<i>Escherichia coli</i> (n = 2)	24.4 ±3.9	18.7±4.8	9.7±1	8.2±0.3
	* <i>Klebsiella pneumoniae</i> (n = 2)	12.3±6.7	7.3±7.02	0	0
	<sup>†</sup> <i>Proteus mirabilis</i> (n = 1)	11.3±3.2	3±5.2	0	0
Enhancing	<i>Escherichia coli</i> (n = 2)	0	0	0	0
	<i>Klebsiella pneumoniae</i> (n = 2)	32.9±9.4	27.3±8.2	24.2±5.1	17.5±5.3
	<i>Proteus mirabilis</i> (n = 1)	25.3±13.9	20.7±6.6	12.8±4.9	8.9±3.6

\*Effect on Si, Mb, and Tl only

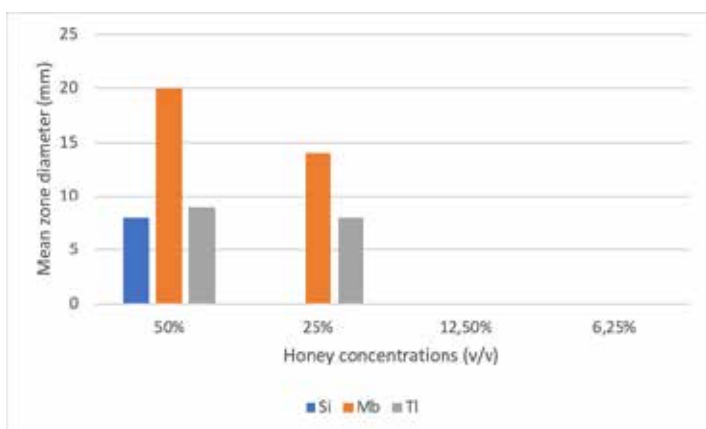
<sup>†</sup>Effect on Sp, Mb, and Tl only

**Discussion**

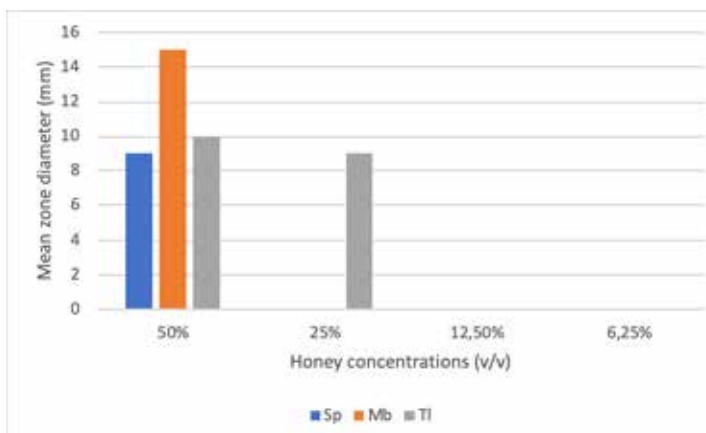
Honey has been in use to treat various human diseases [3-5]. Despite being widely accepted for its wide therapeutic potential, its applications are still limited to CAM. Microbiological studies have reported antimicrobial properties of honey, but the lack of scientific research on the mode of action on various pathogens has not allowed honey to access a place in modern medicine [6, 7]. The majority of studies have settled the inhibitory effect responsibility on the hydrogen peroxide content of honey [10, 14-16]. Researchers have recently reported on the contribution of non-hydrogen peroxide agents to the antimicrobial action of honey as well [7]. A number of microbiological research studies have shown antimicrobial property of honey types [9-13]. The present study found that the honey has not only inhibitory but also enhancing effect on bacterial growth. Our study observed the inhibitory effect on *E. coli* in all types of honey studied, whereas on *K. pneumoniae* and *P. mirabilis* in just three types of honey with 25% and 50% concentrations. The antibacterial effect in our honey samples is consistent with other studies. Although all the honey types have an inhibitory effect on *E. coli*, 25% and 50% concentrations reflect remarkably large inhibitory zones. The inhibitory effect was observed proportionally with decreasing concentration. However, the inhibitory effect of Sw honey was seen almost equally at 50%



**Figure 1.** Mean inhibitory effect of all honey types on *E. coli* (n = 2)



**Figure 2.** Mean inhibitory effect of honey types on *Klebsiella pneumoniae* (n = 2)



**Figure 3.** Mean inhibitory effect of honey types on *Proteus mirabilis* (n = 1)

and 25% concentrations for *E. coli* inhibition. Bm and Mb were very effective at 50% concentration to inhibit *E. coli*. The H<sub>2</sub>O<sub>2</sub> concentration in *Acacia* honey is reported lesser than that in *Ziziphus* honey [19]. The Sw honey is *Acacia* honey that has shown a retained similar inhibitory effect on *E. coli* at 50% and 25% concentrations, whereas the antibacterial potential of Si (*Ziziphus*) has been found to be lagged or equal in activity compared to Sw honey (*Acacia*) (Figure 1). This observation indicates the activity of another H<sub>2</sub>O<sub>2</sub> independent antibacterial mode of action in Sw honey, which remained uninfluenced at 50% and 25% concentrations.

Our study has found that 50% concentration of Bm (clover) honey showed the maximum inhibitory effect among all 9 types of honey studied. Researchers have reported the H<sub>2</sub>O<sub>2</sub> quantity in clover honey and *Ziziphus* honey in their separate studies [11, 14, 19]. It can be noticed from these studies that H<sub>2</sub>O<sub>2</sub> in clover honey is lesser than Si honey (*Ziziphus*). Also, Sm and Sw (*Acacia*) showed a similar inhibitory effect as Si honey. This finding supports that honey has some other mechanism of action than only of H<sub>2</sub>O<sub>2</sub>. We are of the opinion that along with H<sub>2</sub>O<sub>2</sub>, a combined inhibitory effect of other ingredients of honey might be observed. All bacterial isolates studied in the present study were catalase-producing organisms. This might be one of the reasons that H<sub>2</sub>O<sub>2</sub> could not exhibit a noticeable effect.

On *Klebsiella* isolates, Si honey showed an inhibitory zone followed by enhancing zone (double zone) at 50% concentration. TI honey gave the same effect of the double zone at 50% and 25% concentrations. The double zone was also observed in *Proteus* isolates with 50% concentration of Sp honey and with 50% and 25% concentrations of TI honey. Mb was found inhibitory at 50% and 25% concentration for the isolates of *Klebsiella* and 50% concentration only for *Proteus*. Sp honey was observed ineffective at 6.25% and 12.5% concentrations for *Proteus*. Rest all honey types at respective concentrations gave only enhancing effect on *Klebsiella* and *Proteus*. These observations showed that the honey effect is also related to concentration. At lower concentrations, it is less inhibitory and more enhancing. Looking over the overall inhibitory effect of all honey types on the organisms studied, it can be observed that the honey concentration influences the antimicrobial potential (Figures 1-3). However, the pattern of inhibitory effect in the figures reveals a dissimilar varying antimicrobial efficacy in the honey types in respective concentration groups. This effect might be due to the individual antimicrobial efficacy of the honey ingredients at varying concentrations. This also supports that the antimicrobial effect of honey is a combined inhibitory action of responsible ingredients.

### Conclusions

The antimicrobial action of honey on microbes has been a major focus of researchers. The important finding of our study is the growth-enhancing effect of all types of honey on *Klebsiella* and *Proteus* isolates. The results suggest varying effects of honey on bacterial isolates depending on the botanical source of origin, concentration, and type of bacteria. The antimicrobial activity in honey is shown to be due to its hydrogen peroxide content. Of the honey samples in the present work, *Acacia*, *Ziziphus*, and clover honey are known for their hydrogen peroxide quantity. Honey of *Ziziphus* origin has higher H<sub>2</sub>O<sub>2</sub> than *Acacia* and clover.

Clover honey (Barseem) with maximum inhibitory effect on *E. coli* among all honey types, weaker or equal inhibitory potential of *Ziziphus* honey than *Acacia* honey, and a similar inhibitory effect between 50% and 25% concentrations of the same *Acacia* honey (Shawkah) are the observations in this study, which signals the presence of some other additional H<sub>2</sub>O<sub>2</sub> independent mechanisms in these types of honey. With this, we consistently state the inhibitory effect owing to the combined antimicrobial potential of honey compounds instead of H<sub>2</sub>O<sub>2</sub> alone. Our findings also suggest that the effect of honey is influenced by the dilution; at lower concentration, it is more enhancing and less inhibitory to the growth. On *Klebsiella* and *Proteus*, only Tualha and Majra Baldee had inhibitory action on isolates at 50% and 25% concentrations, whereas the rest of all concentrations and honey types have shown a growth-enhancing effect. The varied inhibitory and enhancing effects of honey types in the present study reflect the prebiotic property of honey which requires further microbiological studies to pave the way to support the prebiotic nature of honey.

### Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

### Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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### Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

### References

- Alqarni AS, Hannan MA, Owayss AA, Engel MS. The indigenous honey bees of Saudi Arabia (Hymenoptera, Apidae, *Apis mellifera jemenitica* Rutner): their natural history and role in beekeeping. *Zookeys*. 2011; (134): 83-98.
- Alvarez-Suarez JM, Gasparini M, Forbes-Hernández TY, Mazzoni L, Giampieri F. The composition and biological activity of honey: a focus on manuka honey. *Foods*. 2014; 3(3): 420-32.
- Miguel MG, Antunes MD, Faleiro ML. Honey as a complementary medicine. *Int Med Insights*. 2017; 12: 1-15.
- Eteraf-Oskouei T, Najafi M. Traditional and modern uses of natural honey in human diseases: a review. *Iran J Basic Med Sci*. 2013; 16(6): 731-42.
- Alrowais NA, Alyousefi NA. The prevalence extent of complementary and alternative medicine (CAM) use among Saudis. *Saudi Pharm J*. 2017; 25(3): 306-18.
- Carter DA, Blair SE, Cokcetin NN, Bouzo D, Brooks P, Schothauer R, et al. Therapeutic manuka honey: no longer so alternative. *Front Microbiol*. 2016; 7(569): 1-11.
- Roberts AEL, Brown HL, Jenkins RE. On the antibacterial effects of manuka honey: mechanistic insights. *Res Rep Biol*. 2015; 6: 215-24.
- VanKetel BA. Festnummer der Berichten van den Niederlandische Maatschappij. *Bevordering der Pharmacie*. 1892; 67-96.
- Ghramh HA, Khan KA, Alshehri AMA. Antibacterial potential of some Saudi honeys from Asir region against selected pathogenic bacteria. *Saudi J Biol Sci*. 2019; 26(6): 1278-84.
- Bucekova M, Jardekova L, Juricova V, Bugarova V, Marco GD, Gismond A, et al. Antibacterial activity of different blossom honeys: New findings. *Molecules*. 2019; 24(8):1573. DOI:10.3390/molecules24081573.
- Elbanna K, Attalla K, Elbadry M, Abdeltawab A, Gamal-Eldin H, Ramadan MF. Impact of floral sources and processing on the antimicrobial activities of different unifloral honeys. *Asian Pac J Trop Dis*. 2014; 4(3): 194-200.
- Mandal S, Mandal MD, Pal NK, Saha K. Antibacterial activity of honey against clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* serovar Typhi. *Asian Pac J Trop Med*. 2010; 3(12): 961-4.
- Mandal MD, Mandal S. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed*. 2011; 1(2): 154-60.
- Brudzynski K, Abubaker K, St-Martin L, Castle A. Re-examining the role of

hydrogen peroxide in bacteriostatic and bactericidal activities of honey. *Front Microbiol.* 2011; 2(213): 1-9.

15. Brudzynski K. Effect of hydrogen peroxide on antibacterial activities of Canadian honeys. *Can J Microbiol.* 2006; 52(12): 1228-37.

16. Weston RJ. The contribution of catalase and other natural products to the antibacterial activity of honey: a review. *Food Chem.* 2000; 71(2): 235-9.

17. Louveaux J, Maurizio A, Vorwohl G. International commission for bee botany of IUBS. *Methods of melissopalynology.* Bee Word. 1978; 59(4): 139-57.

18. Al Somal N, Coley KE, Molan PC, Hancock BM. Susceptibility of *Helicobacter pylori* to the antibacterial activity of manuka honey. *J R Soc Med.* 1994; 87(1): 9-12.

19. Mohammed MEA, Alaergani W, Suleiman MHA, Al-Graham HA. Hydrogen peroxide and dicarbonyl compounds concentration in honey Samples from different botanical origins and altitudes in the South of Saudi Arabia. *Curr Res Nutr Food Sci.* 2019; 7(1). DOI: <http://dx.doi.org/10.12944/CRNFSJ.7.1.15>.

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